American Journal of Hematology 58:16–19 (1998)

Factors Affecting the Rate of Iron Mobilization During Venesection Therapy for Genetic Hemochromatosis

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Although progressive iron accumulation is a characteristic feature of genetic hemochromatosis, the factors affecting the rate of iron mobilization by venesection have not been established. Venesection records were analyzed in 77 hemochromatosis homozygotes to study the factors affecting the rate of iron mobilization by venesection. The rate of iron mobilization was the iron removed divided by the time required to deplete iron stores (serum ferritin < 50 μg/L). Mean duration of venesection therapy was 1.4 years (range 0.44–3.6 years). All patients completed the therapy and there were no significant adverse effects. Rate of iron mobilization was higher in cirrhotics compared to non-cirrhotic patients ($P = 0.04$). Iron mobilization was inversely related to intestinal radioiron absorption ($r = -0.45$, $P = .01$). There was no significant relationship between iron mobilization and patient age, gender, serum ferritin, and hepatic iron concentration. Iron mobilization is increased in cirrhotics and patients with lower intestinal iron absorption. Venesection therapy is safe and well tolerated in all age groups. Am. J. Hematol. 58:16–19, 1998.

Key words: venesection therapy; genetic hemochromatosis; iron mobilization

INTRODUCTION

Genetic hemochromatosis is recognized as a disease characterized by the progressive accumulation of iron. However, the use of venesection therapy to remove iron followed the clinical description of the disease by 50 years [1]. A candidate gene has been described on chromosome 6 but the mechanism of iron accumulation has not been established [2]. Erythropoiesis stimulated by venesection therapy leads to the mobilization of iron from parenchymal cells. Duration of venesection therapy is longer in more advanced disease but the factors affecting rate of iron mobilization in hemochromatosis have not been clearly established.

METHODS

Clinical records were available on 77 hemochromatosis patients that had their entire venesection therapy at this hospital. Since this is a tertiary referral center for hemochromatosis, there were many homozygotes that had periodic venesection therapy at another medical center.

The diagnosis of hemochromatosis was based on clinical history, physical examination, liver biopsy, and family investigations. All patients had either a hepatic iron index (hepatic iron index > 1.9) or greater than 5 g removed by venesection. Genetic testing for the C282Y mutation of the HFE gene on chromosome 6 was performed on 50 of the patients in this study. PCR amplification (forward primer: 5′GGCAAGGGTAAACAGATCC3′ and reverse primer: 5′CTCAGGCACTCCTCTCAACC3′) generates a product of 387 bp size. When digested with the restriction enzyme RsaI, normal DNA generates two fragments of 247 and 140 bp size. In a homozygote, the mutant DNA, due to creation of a new restriction site secondary to the mutation, generates a total of three fragments with 247, 111, and 29 bp size. In heterozygotes, due to the presence of both normal and mutated DNA, a total of four fragments with 247, 140, 111, and 29 bp are produced. Patients can be as-

Contract grant sponsor: Canadian Liver Foundation; Contract grant sponsor: PSI Foundation of Ontario.

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Received for publication 10 April 1997; Accepted 10 December 1997

TABLE I. Clinical and Biochemical Profile of 77 Hemochromatosis Patients*

Gender	64 men, 13 women
Age	53 ± 13 years*
Serum ferritin (15–300 μg/L)	2,554 ± 2,123 μg/L
Mean hemoglobin at the end of venesection therapy	13.6 ± 1.4 g/dl
C282Y homozygotes	47/51 (92%)
Liver iron concentration (n = 37, 0–35 μmol/g)	393 ± 195 μmol/g
Hepatic iron index (n = 37, normal < 2)	7.7 ± 4.1
Iron removed (g)	11.5 ± 6.0 g
Cirrhosis	11/77 (14%)
Iron absorption (n = 26)	52 ± 29%
Serum erythropoietin (n = 11, 9–30 U/L)	17 ± 8.5 U/L
Iron accumulation rate	0.25 ± 0.19 g/year
Iron mobilization rate	9.4 ± 3.1 g/year = 1 venesection every 1.38 weeks

*Data presented as mean ± standard deviation.

signed to be homozygous, heterozygous, or normal for this mutation. Putative homozygotes that were not homozygous for the C282Y mutation were tested for a second mutation (H63D) as previously described [2–4]. There were no patients with a history of iron loading anemia or multiple transfusions. Patients were initially treated by the weekly removal of 500 ml of blood. Patients attended an ambulatory care facility and the venesection was performed by a nurse using a kit containing a 16-gauge straight needle and collection bag (Blood Pack MR6102, Baxter, Deerfield, IL). Blood was removed with the patient in the reclining position over 15–30 min. A hemoglobin was done at the time of each venesection. If the hemoglobin decreased to less than 10 g/dl the venesection schedule was modified to 500 ml every other week. Venesections were not routinely done during patient vacations away from this medical center. Venesections were continued until the serum ferritin was approximately 50 μg/L. Maintenance venesections after iron depletion of 3 to 4 venesections per year were not included in this study. Iron removed was estimated by the number of venesections × 0.25 g. The rate of iron accumulation was defined as the iron removed (g) divided by the age at diagnosis and the rate of iron mobilization was the iron removed divided by the time required to deplete iron stores. Radioiron intestinal absorption studies were available on 26 of these patients that had been previously studied as part of another study on iron absorption and hemochromatosis [5]. Iron absorption was measured at the time of diagnosis using total body counting following the oral ingestion of 1 μCi of ^{59}Fe citrate administered with 10 μmol of unlabelled ferrous ascorbate. The absorption was calculated as the percentage of the initial dose retained at 14 days [5]. Hepatic iron concentration was measured by atomic absorption spectrophotometry and the hepatic iron index was the hepatic iron concentration/age [6]. Serum erythropoietin (EPO) was measured 2 months after the commencement of venesection therapy in 11 patients just prior to the next weekly venesection. Since the levels in all patients were in the normal range, no further studies were done on EPO in hemochromatosis. HLA A and B testing done on all hemochromatosis homozgotes. Iron mobilization rates were compared between age groups using ANOVA and between men and women using the Student's *t* test. Pearson correlation coefficients were calculated for the relationship between clinical factors and rates of iron accumulation and mobilization.

RESULTS

There were 51 patients that had genetic testing done. Forty-seven (92%) were homozygotes for the C282Y mutation. One heterozygote was classified clinically as juvenile hemochromatosis with severe cardiac involvement, and there were 3 cases of iron overload (hepatic iron index > 1.9) that were negative for both the C282Y and H63D mutation. Of the 26 patients that did not have genetic testing, 15 had an affected sibling with iron overload. The clinical profile of the 77 hemochromatosis patients is shown in Table I. The relationship between the clinical factors and the rates of iron accumulation and mobilization is shown in Table II. A correlation was demonstrated between the rate of iron accumulation and liver iron concentration and the rate of iron mobilization and radioiron absorption ($r = -0.45$, $P = .01$). Serum EPO was in the normal range in all patients tested (17 ± 8.5 U/L, normal range = 9–30, n = 11). There were no differences in the iron accumulation or mobilization rates between patients with 0 (n = 20), 1 (n = 46), and 2 (n = 11) haplotypes with HLA-A3. There were no differences in the iron mobilization rates between men and women and between age groups as quartiles (Fig. 1). There were no patients that could not tolerate the venesection therapy. Complications included local phlebitis

TABLE II. Correlation of Clinical Factors With Iron Accumulation and Mobilization in Hemochromatosis*

Clinical factor	n	Iron accumulation rate	Iron mobilization rate
Age	77	N/A	r = .02, P = 0.44
Serum ferritin	77	r = 0.44, P < .0001	r = −0.14, P = 0.14
Liver iron concentration	37	r = 0.5, P < .0001	r = −0.21, P = 0.1
Radioiron absorption at diagnosis	26	r = 0.3, P = .08	r = −0.45, P = .01
Mean hemoglobin during therapy	77	N/A	r = 0.35, P = .001
Serum EPO during therapy	11	N/A	r = 0.08, P = 0.41
Iron accumulation	77	—	r = 0.1, P = 0.18

*The iron accumulation rate incorporates the age of the patient. EPO = erythropoietin; N/A = not applicable.

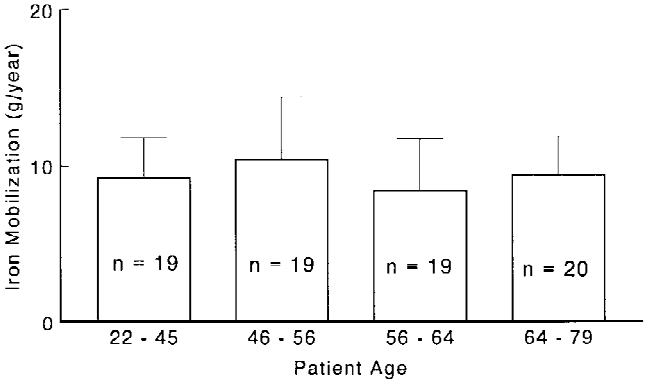

Fig. 1. The relationship between the iron mobilization rate and age of the patient at diagnosis.

and lightheadedness, which was managed by the self-administration of a salt-containing soft drink.

DISCUSSION

Although there have been no randomized clinical trials of venesection therapy in hemochromatosis, there have now been many studies that have demonstrated an improvement in symptoms and long-term survival after venesection therapy [1,7–11]. The periodic removal of blood stimulates erythropoiesis and iron is mobilized from parenchymal organs to support the synthesis of new blood cells. The cellular mechanisms involved in iron release from parenchymal cells in vivo have not been established in hemochromatosis [12,13]. The rate of iron accumulation and tissue damage in hemochromatosis has been known to be highly variable [11,14–16]. In this study, the coefficient of variation for iron accumulation was 79% and for mobilization 33%. There was no obvious relationship between rate of iron accumulation and rate of iron mobilization. The rate of accumulation was associated with the liver iron concentration. This would be consistent with previous studies that have demonstrated a relationship between the iron removed by venesection and liver iron concentration [17]. The observation that the rate of iron mobilization is inversely related to iron absorption is also consistent with previous studies demonstrating an inverse relationship between iron absorption and hepatic iron concentration in hemochromatosis [5]. Since iron absorption is constantly changing throughout the course of hemochromatosis and during venesection therapy, both the iron accumulation and mobilization rates are oversimplifications of a complex homeostatic relationship between iron stores, intestinal iron absorption, iron excretion, and erythropoiesis [18]. This study did not demonstrate a relationship between a lower mean hemoglobin or elevated serum EPO level and rate of iron mobilization. Previous studies in iron-loaded rats have demonstrated increased hepatic iron mobilization with exogenous administration of EPO [19]. The patients in this study did not have evidence of anemia at the time that they reached the target serum ferritin of 50 μg/L [20]. Alternative therapeutic approaches in hemochromatosis have used the development of anemia or a fall in transferrin saturation as the end point of venesection therapy [1,21,22]. We do not recommend repeat liver biopsy to document the presence of iron depletion.

There was no evidence of increased iron accumulation or mobilization in patients with haplotypes containing HLA-A3. This contrasts with the study of Piperno et al. [23] in which patients with the ancestral haplotype including HLA-A3 demonstrated more evidence of iron overload. Many patients in the Italian study group had other factors, which may affect iron balance in the liver including chronic viral hepatitis and alcoholism [23].

This study demonstrated that elderly patients could tolerate venesection therapy as well as younger patients. The side-effect profile and cost of venesections compare favorably to potential therapies with oral iron chelating agents such as deferiprone. Iron mobilization is increased in cirrhotics and patients with lower intestinal iron absorption. The rate of venesection therapy can be easily monitored by the simple measurement of the hemoglobin weekly and continued until the ferritin is approximately 50 ug/L. New developments in molecular genetics will advance our understanding of genetic hemochromatosis,

but the medieval therapy of periodic venesection will likely prevail as the preferred therapy.

ACKNOWLEDGMENTS

The author acknowledges the advice and assistance of Leslie Valberg, Ann Kertesz, and Subrata Chakrabarti, and the generous support of the Pomajba family.

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